Chiron Dkt. No.: 19720.004
(Atty Dkt. No. 072121-0274)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Erwin *et al.*

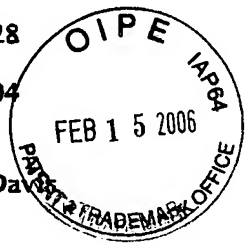
Title: ANTIBACTERIAL AGENTS

Appl. No.: 10/754,928

Filing Date: 01/08/2004

Examiner: Brian J. Davis

Art Unit: 1621



I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class mail in an envelope addressed to: Mail Stop _____, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 13 day of February, 2006.

By _____

DECLARATION OF ERIC HARWOOD UNDER 37 C.F.R. § 1.132

Dear Examiner Davis:

I, DR. ERIC HARWOOD, state and declare that:

1. I am the inventor of the invention recited in at least claims 25-27. At the time of my invention, I was an employee of Pathogenesis, now Chiron.

2. Before the publication of the cited art, Kline et al., *Journal of Medicinal Chemistry* (2002), 45(14), p. 3112-29, on June 7, 2002 (Web publication date), I conceived of the subject matter of at least claims 25-27 and 32 as evidenced by the attached Exhibits A and B.

3. Exhibit A includes copies of representative notebook pages documenting the synthesis of various compounds which are amino acid derivatives bearing a hydroxyalkyl side chain, a hydroxamic acid group, and a hydrophobic N-acyl group. Each of these compounds was synthesized for testing as an inhibitor of LpxC prior to June 7, 2002. Pages 35 and 42 show the initial synthesis of an exemplary compound of the claims. Pages 73, 77, 80, 83 and 90 show the synthesis of other representative compounds as part of library 1a. I conceived and synthesized each of the compounds in library 1a.

4. Exhibit B is an excerpt from an internal research report I prepared prior to June 7, 2002. The report documents the structures and synthesis of hydroxamic acids which I had prepared and proposed to prepare at the time as LpxC inhibitors. Among the compounds I prepared were compounds of library 1a which I had synthesized by the time of the report. The report also documents the compounds of library 2a which I had conceived of and knew how to make by the time of the report. Each "acyclic" compound (as distinguished

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from cyclic oxazoline compounds) of libraries 1a and 2a is an amino acid derivative bearing a hydroxyalkyl side chain, a hydroxamic acid group, and a hydrophobic N-acyl group.

5. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the above-referenced application or any patent issuing thereon. All statements made of declarant's own knowledge are true and all statements made on information and belief are believed to be true.

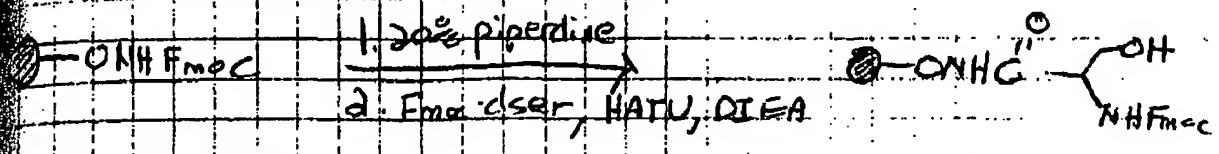
E. Harwood — 13Feb06

Eric Harwood, Ph.D. Date

Exhibit A

coupling

No.



	dser	HATU	DIEA	50% DMF/CH2Cl2
MW	1 mmol/g	327.3	380.2	129.25 (d = .742)
amount	500 mg	307.3 mg	380.2 mg	348 ~~ml~~ 192 ul 10 ml
mmol	.5 mmol	1 mmol	1 mmol	~~1 mmol~~ 1.1 mmol

Resin was swelled for .5 hours in CH_2Cl_2. Treat with 10 ml 20% piperdine/DMF for 1 hour, Drain repeat 2 more times. Rinse 1X DMF, 3X CH_3OH, 3X CH_2Cl_2. In seperate flask dissolve dser in 10 ml 50%DMF/CH_2Cl_2 add DIEA and cool to 0°. Add HATU in 1 portion Stir at 0°C for ½ hour. Add to Resin via Syringe. Shake overnight.

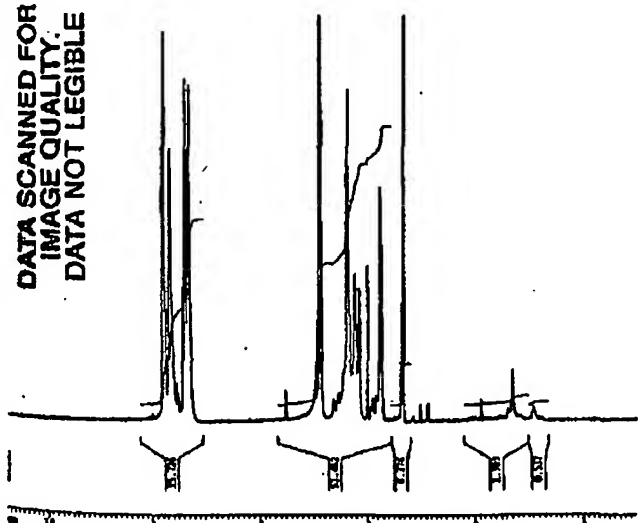
~~Rinse 1X DMF, 3XCH_3OH, 3XCH_2Cl_2~~. HPAC → 768-35 → 569mg

~~take 50mg and cleave w/ TFA~~ → 768-35-1 → 29mg

↓

25 mg after HPAC, overnight

↓ purify PTLC 7% meoh/CH_2Cl_2



768-35-2 → 12.7mg pure

not soluble in acetone, good recryst?

To Page No.

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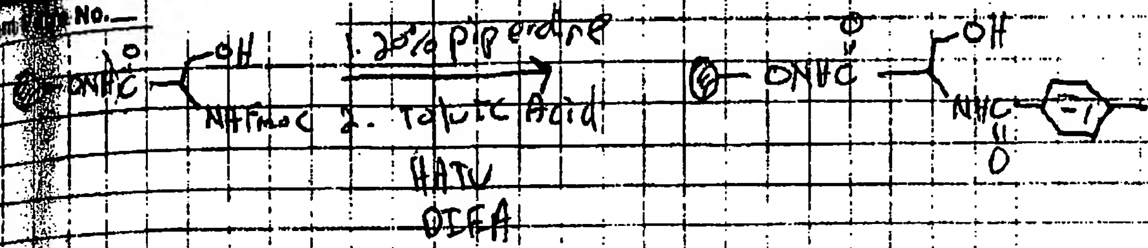
Recorded by

Project No.

Book No. 768 TITLE Coupling

No.



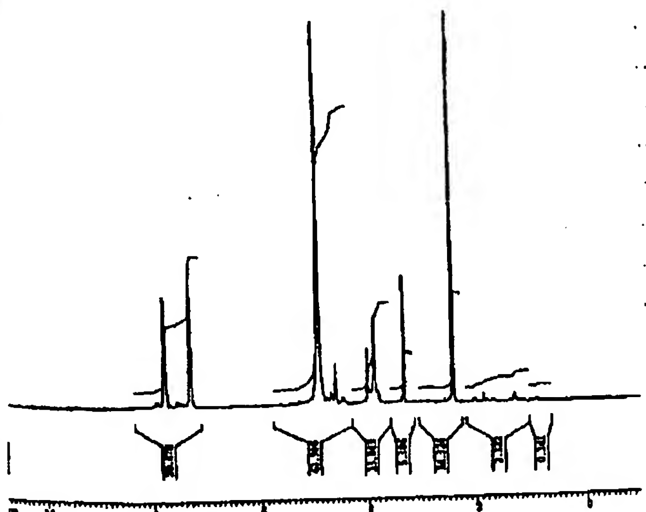
	768-35	toluic	HATU	DIEA
MW	.75mmol/g	136.15	380.2	129.25(d=.742)
amount	400mg	82 mg	228 mg	122 ul
mmol	.300 mmol	.600 mmol	.600 mmol	.700 mmol

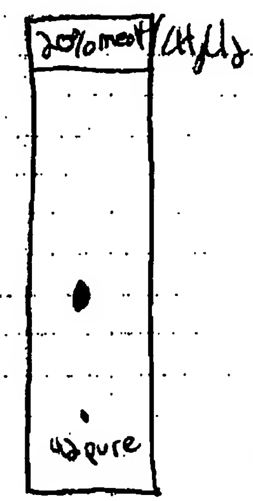
swell CH_2Cl_2 ½ hour. Treat 20% piperdine/DMF 3x 1 hour each. Rinse DMF, CH_3OH, CH_2Cl_2. Dissolve Toluic Acid in 8 ml 50% DMF/CH_2Cl_2 and DIEA cool to 0°C. Add HATU stir 0°C ½ hour. Add to Resin. Stir overnight. Rinse DMF, CH_3OH, CH_2Cl_2.

Cleave 50 mg in TFA. → 768-42 purify PTLC 20% MeOH CH_2Cl_2. obtain ~ 7.5 mg → 768-42-2

HPLC & MS are good

.61 mmol/g





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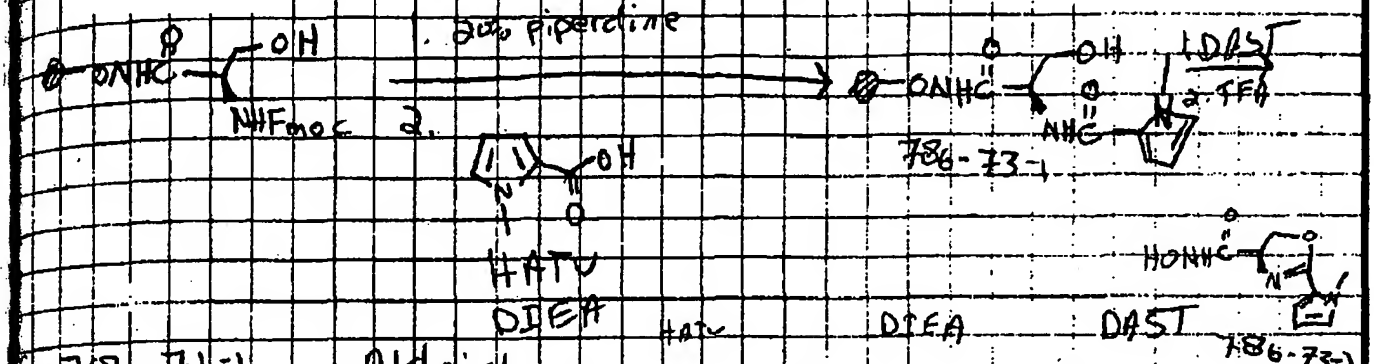
NB76800

TLE Library 1a

Page No. 71



768-71-1	Aldrich					
M.W. assume 1mmol/g	125.13	380.2	129.25 (d=.742)	161.19 (d=1.22g)		
Amount 450mg	85mg	257mg	160 µl	150 µl		
mmol .45 mmol	.675 mmol	.675 mmol	.90 mmol			

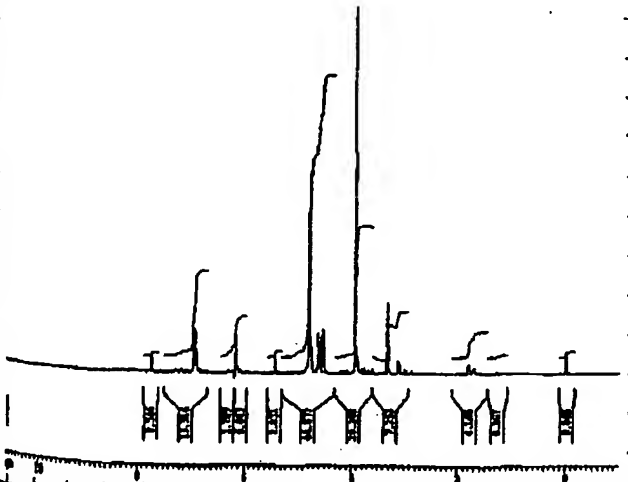
#1 Swell in CH_2Cl_2 5 hour. Treat 3x w/ 20% piperdine/DMF 1 hour each. wash 3x DMF 3x MeOH 3x CH_2Cl_2. Remove 250 mg resin and swell in 5ml CH_2Cl_2 (cool to -30° (cooler setting) add DAST stir 1 hour wash 3x MeOH 3x CH_2Cl_2

768-73-1 → HPLC Shows some impurity at least 80% pure M.S. messy, but shows m+H+

768-73-2 → NMR looks good minor impurities HPLC not great MS great. Submitted as is

→ cleave 10% TFA/CH_2Cl_2 768-73-1 = 4.6 mg

Remaining beads were 768-73-2 = 14.8 mg also cleaved in 10% TFA.



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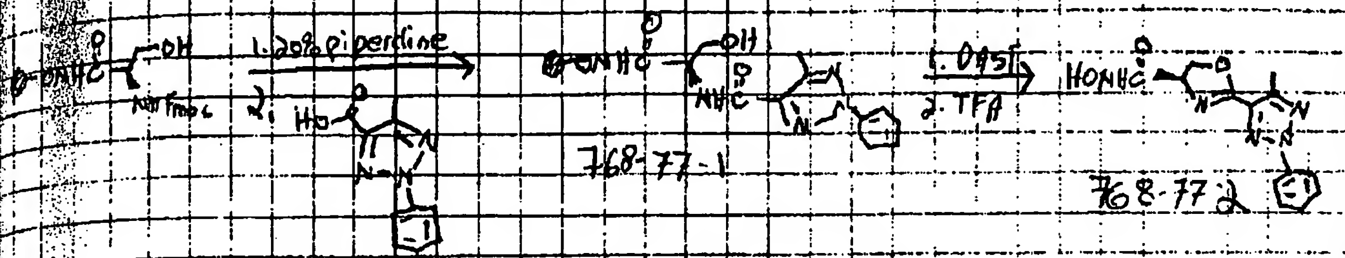
NB76800

TITLE: Oraly 1c

From Page No. 71

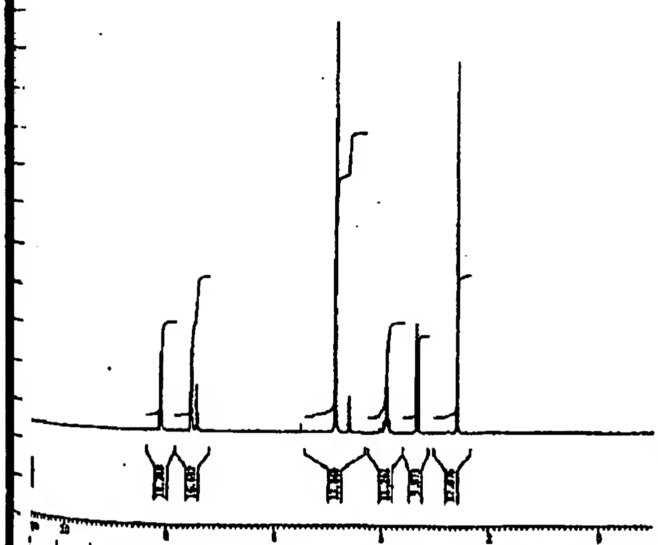


		HATU	DIEA	DAST
S 768-71-1				
M.W. assume 1mmol/g	203.20	380.2	129.25 (d=.Pa2)	
amount 450 mg	137 mg	257	160 ul	
mmol .45 mmol	.675	.675	.90	

~~See 768-73 for prep~~

768-77-1 → HPLC great
M.S. good

768-77-2 → 'H NMR ok
except extra singlet
→ HPLC good
→ M.S. found
M+18



768-77-1 = 12.1mg
768-77-2 = .6mg

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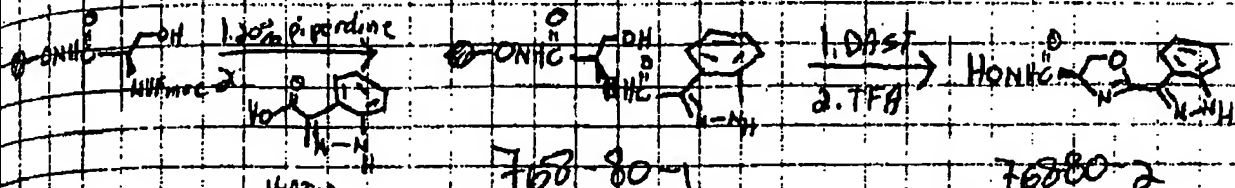
Project No.

Book No. 768 TITLE Library 1a

From Page No. 7



HATU
DIEA

768-80-1

76880-2

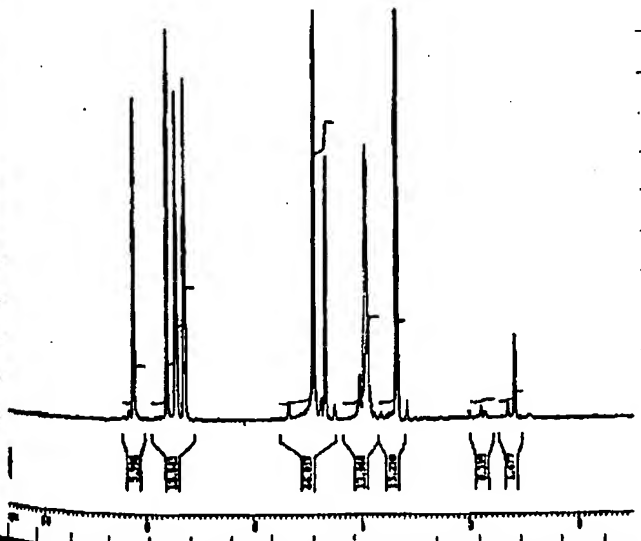
DAST

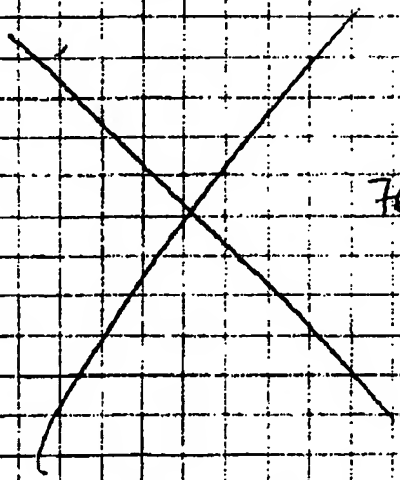
S 768-71-1	Fluka			
Mw: assume 1mmol/g	162.15	380.2	129.25 (74%)	
amount 450 mg	110 mg	257 mg	160 ul	
mmol .45 mmol	.675	.675	.90	

ppt obtained when DIEA added

See 768-73 for prep

768-80-1 → 1HNMR good
HPLC good
M.S. good





768-80-1-9.2

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B76800

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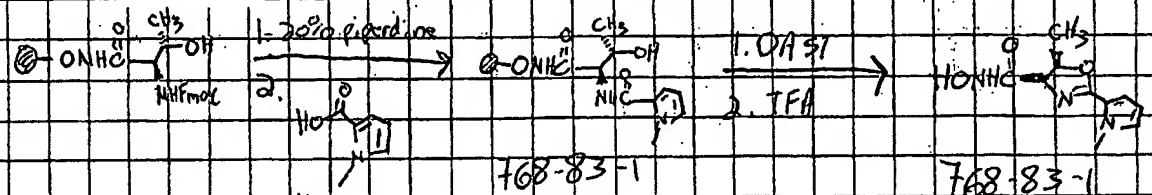


768-83-1

768-83-1

HATU DIEA DAST

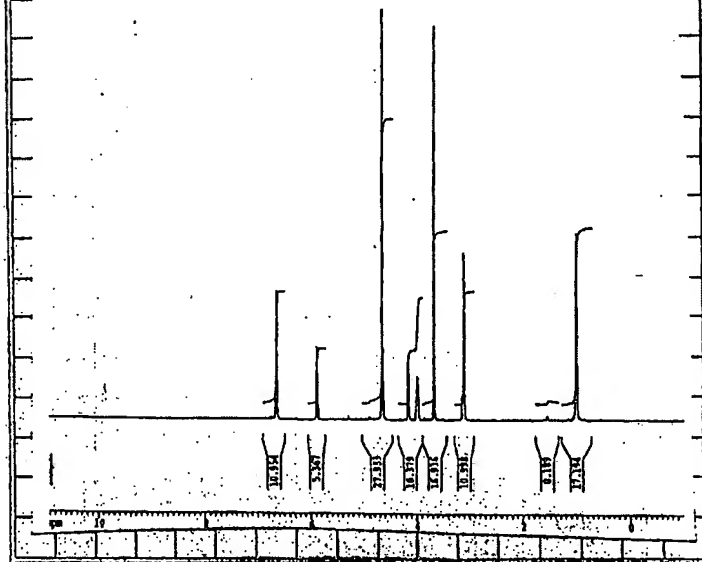
	768-72-1			
M.W. assume 1mmol/g		125.13	380.2	187.25 (.742)
amount	450 mg	85 mg	257 mg	160 ul
mmol	45 mmol	.675	.675	.90

#11

see 768-73 for prep

768-83-1 → 1H NMR good
HPLC good
M.S. found
[M+H]+

768-83-1 = 66 mg



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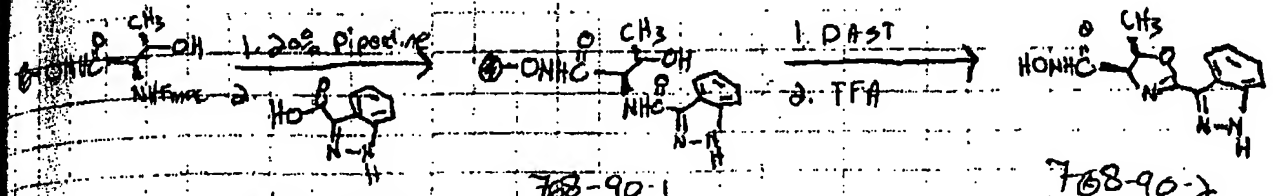
Page No. 7d



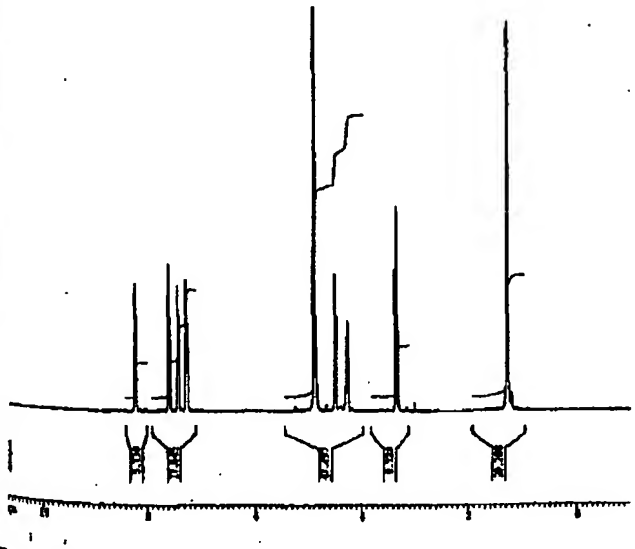
768-90-1 768-90-2

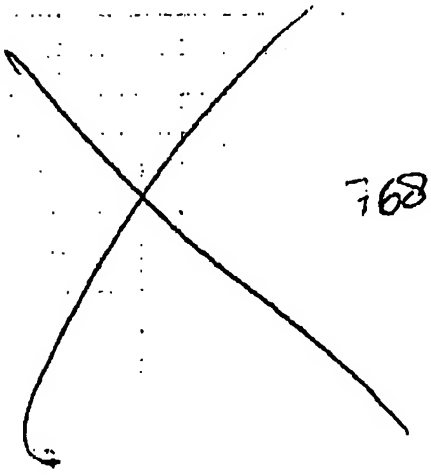
		HATU	DIEA	DAST
768-72-1	Fluka			
M.W. assume 1mmol/g	160.15	380.2	129.25 (.742)	
mass 450 mg	110 mg	257 mg	160 µl	
mmol .45 mmol	.1675	.1675	.90	

pH when DIEA is added

See 768-73 for prep

768-90-1 → 'HMR good
HPLC good
M.S. good





768-90-1 = 15.2mg

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Exhibit B

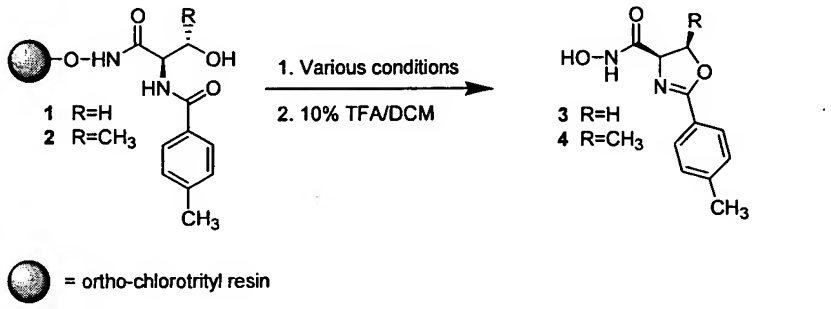
The First Library of LpxC-Directed Hydroxamic Acids

Synopsis

- Optimal conditions for the DAST cyclization were finalized.
- Library 1a was synthesized, purified, and characterized.
- Initial results using TBDMS-protected D-serine for library synthesis were obtained.
- Future Libraries were planned.

Main Points

The first research focus this month was to finalize the optimization of the cyclization of amido alcohols **1** and **2** to the desired oxazolines **3** and **4** (Scheme 1). Last month it was determined that the optimal conditions for conversion of **1** to **3** were 10 equivalents of DAST for 2 hours at temperatures between –20 and -30°C (results are summarized in Table I). However, these conditions resulted in an incomplete reaction and only an 18% yield for the conversion of **2** to **4**. Because of this result, the reaction was performed again on both **1** and **2** using 10 equivalents of DAST at -30°C but this time for a total of 8 hours to push the reaction to completion. It was determined by TLC that only a trace amount of either **3** or **4** was present at the end of 8 hours. Thus, it appeared that the excess DAST reagent was degrading the product over time. To test this hypothesis, a final reaction using **1** (**2** was no longer available in sufficient quantities) and 5 equivalents of DAST at -30°C for only 1 hour was performed. This resulted in a very clean, essentially quantitative conversion of **1** to **3**. We then used these conditions for the synthesis of library 1a.



Scheme 1. Cyclization of Amido Alcohols to Oxazolines

All the reactions were now optimized and the synthesis of the first library of LpxC-directed hydroxamic acids was carried out. The synthesis is outlined in Scheme 2 and has been discussed in previous monthly reports. As we analyzed the structure of the various intermediates on the synthetic pathway, we realized that amido alcohols **12** and **13** had very similar functionality to the desired oxazolines **14** and **15**. The amido alcohols are intermediates on the route to the oxazolines and all that is known about the LpxC binding site is that it requires a zinc binding motif (hydroxamic acid) and a hydrophobic group, thus we decided to screen both the acyclic and oxazoline hydroxamic acids as potential LpxC inhibitors. Therefore, using both D-serine and D-threonine and the 10 nitrogen heterocycles shown in Scheme 2 as the aromatic functionality results in a possible 40 compounds for screening.

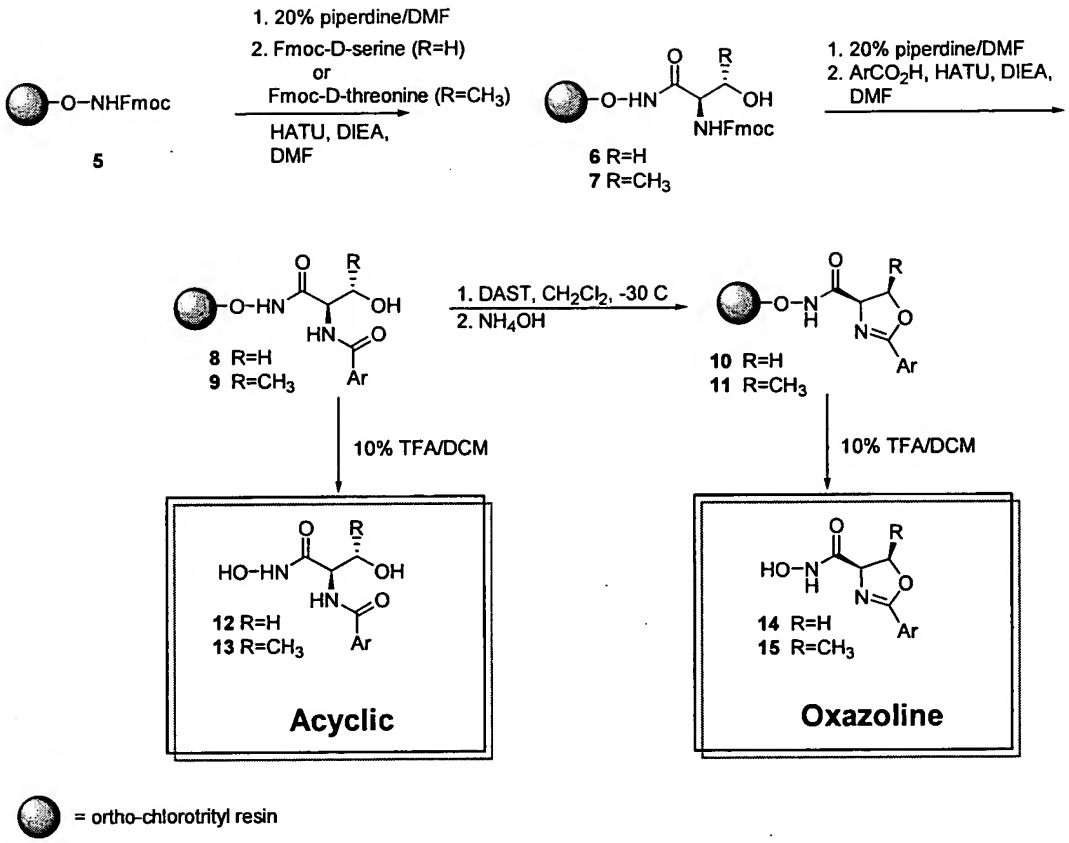
Table I. Optimization of Oxazoline Formation

Amido Alcohol	Product	Conditions	Yield
1	3	DAST (10X), -30°C, 8h	Trace by TLC
2	4	DAST (10X), -30°C, 8h	Trace by TLC
1	3	DAST (5X), -30°C, 1h	100% !
1	3	DAST (10X), -30°C, 2h	35% (very clean)
2	4	DAST (10X), -30°C, 2h	18%* (very clean)
1	3	DAST (10X), -20°C, 2h	44% (very clean)
1	3	DAST (10X), -15°C, 2h	Trace by TLC
2	4	DAST (10X), -15°C, 2h	Trace by TLC
1	3	DAST (10X), 0°C, 2h	0%
2	4	DAST (10X), 0°C, 2h	0%
1	3	$SOCl_2$ (10X), 0°C, 2h	0% (mess)
2	4	$SOCl_2$ (10X), 0°C, 2h	0% (mess)

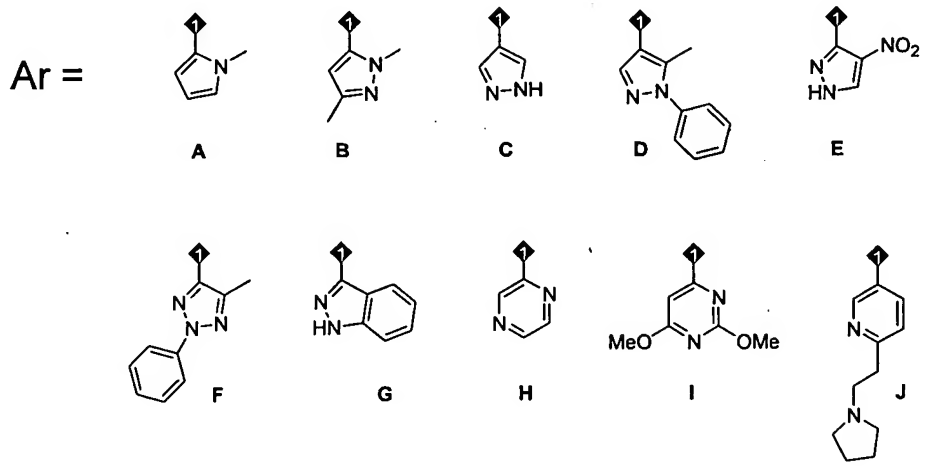
Library 1a was synthesized according to Scheme 2. The crude reaction mixtures were purified by means of pre-packed silica gel columns (1g) except in the case of difficult separations where either preparative scale TLC or HPLC was used as appropriate. Currently, 23 compounds have passed both an identity (MS and/or ^{1}H NMR) and a purity test (HPLC) and are ready for screening (Figure 1). Three more compounds await HPLC purification.

As can be seen in Figure 1, less than the theoretical number of compounds was obtained from library 1a. The synthetic conditions had been optimized using toluic acid as a model, however, the variety of reactivities among the nitrogen heterocycles caused some difficulties. Some of the heterocycles (C, E, and J) did not couple under the conditions determined for toluic acid resulting in no recovery of either the acyclic or oxazoline compound. Most of the other heterocycles that did couple also formed the oxygen/nitrogen bis-acylated product (O-acylation was discussed in the [redacted] report) as well as the desired N-acylated product (**8** and **9**). This not only decreased the yield of desired product, but also made purification quite difficult in some cases.

To avoid the problems associated with O-acylation with the next library, we decided to explore protecting the alcohol of serine as a *t*-butyldimethylsilyl (TBDMS) ether. Fmoc D-serine (**16**) was treated with TBDMS chloride and imidazole in DMF to obtain **17** as illustrated in Scheme 3. The protected D-serine derivative was then coupled to the solid support (**5**) using both HATU and PyBrOP as coupling agents to obtain **18**. TLC analysis of the crude mixtures revealed only one major product (TFA treatment appears to have removed most of the silyl groups). Initial results of the coupling reaction with **17** appear to indicate that it is an improvement over the mixtures obtained when using **16**. Using **17** will allow us to using a more powerful coupling agent (e.g. PyBrOP) and more equivalents of acid to get a higher coupling efficiency without worry of O-acylation. Before Library 1b is synthesized using **17**, the synthesis will be completed with toluic acid to validate the coupling in the presence of the TBDMS group as well as removal of the TBDMS group with tetrabutylammonium fluoride (TBAF).



Library 1a (nitrogen heterocycles)



Scheme 2. Synthesis of Library 1

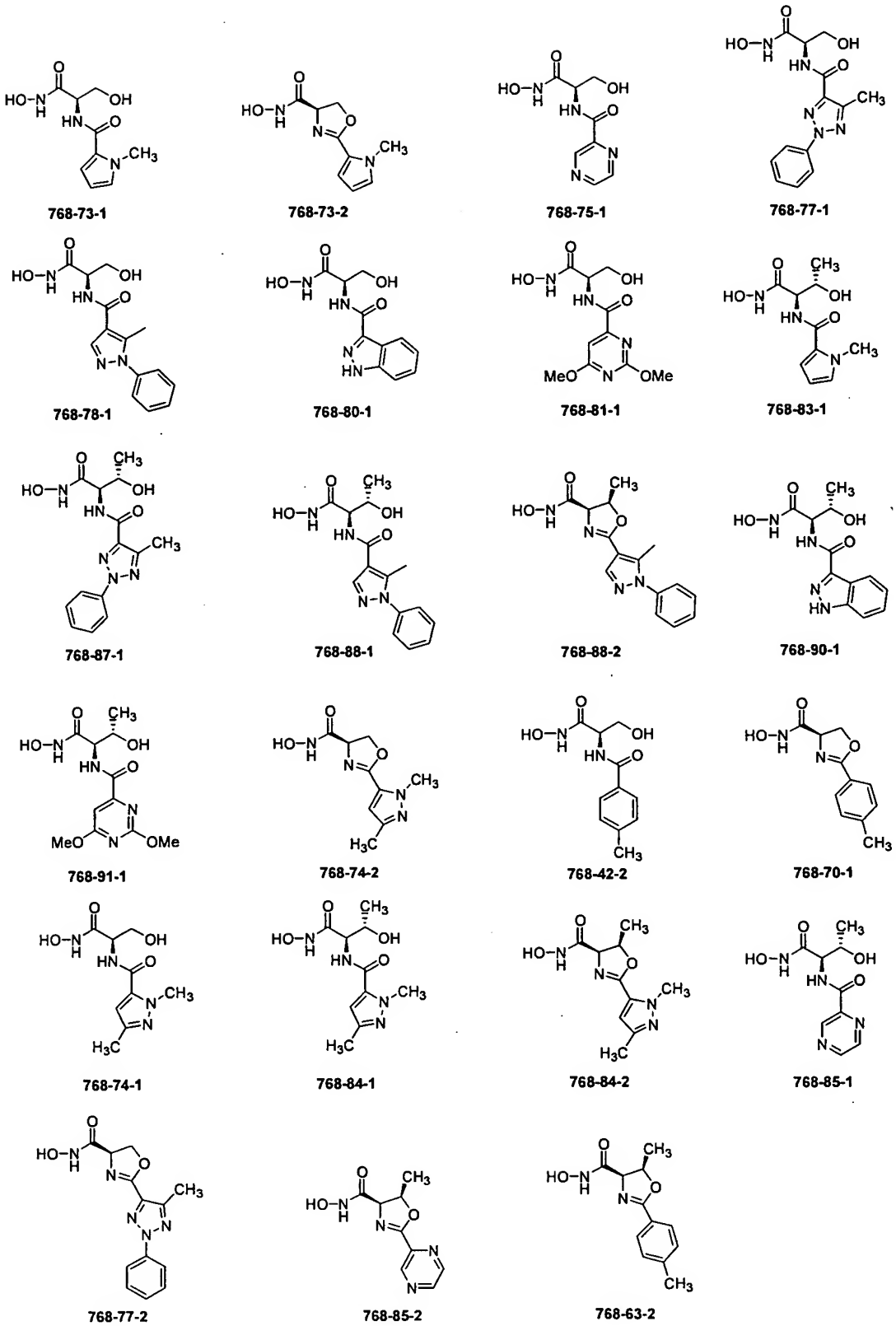
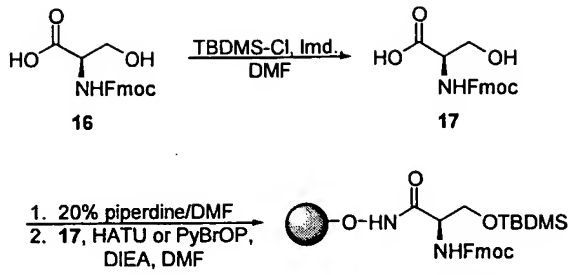


Figure 1. Compounds Ready for Screening



Scheme 3. Synthesis of and Coupling with a TBDMS Protected Serine

Library 1b (Figure 2) has been planned and will be carried out in the near future. For this second library we have chosen 20 aromatic acids representing two different types of compounds. The first ten acids have predominately hydrophobic contacts with long hydrocarbon chains to mimic that of the natural substrate for LpxC. The second ten acids represent a group with predominately polar and hydrogen bonding interactions. This library should give us some good SAR information and will carried out in the next week after the synthesis with **17** is validated.

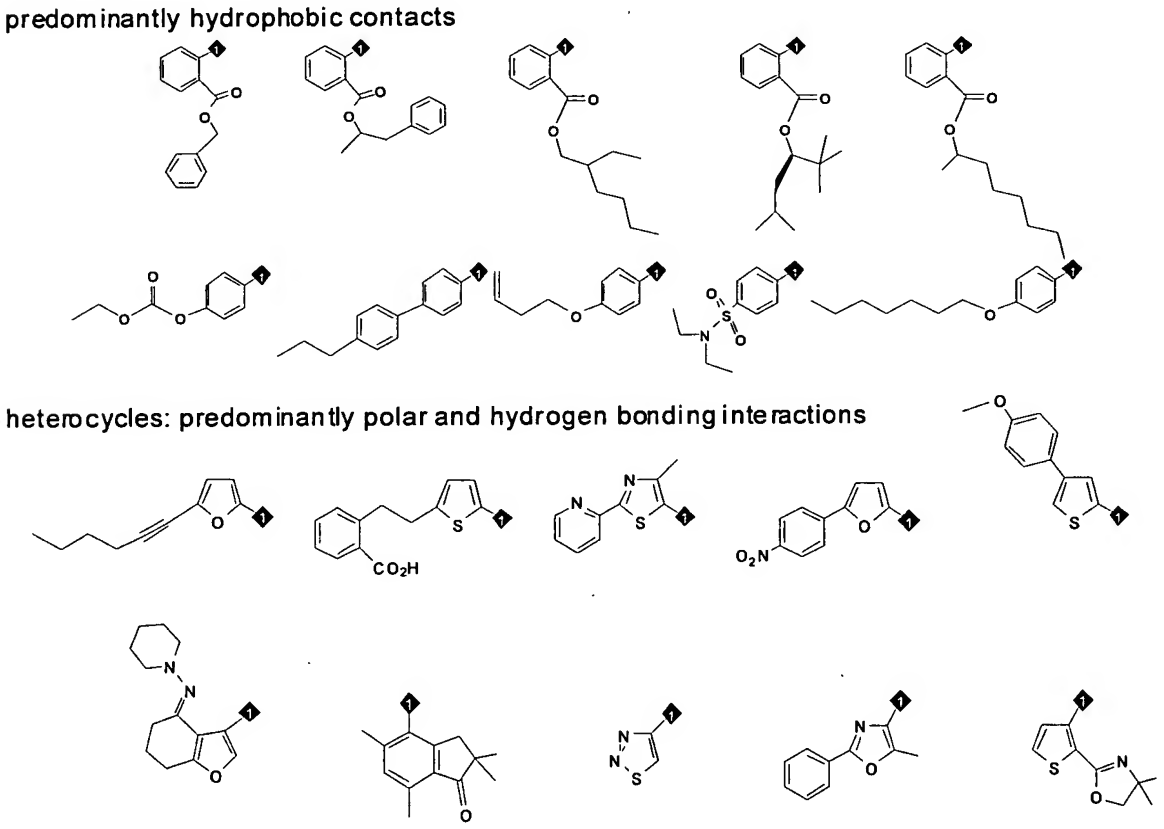


Figure 2. Proposed Aromatic Groups for Library 1b